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Report of the FAO/UNEP

MEETING ON THE TOXICITY AND BIOACCUMULATION OF SELECTED SUBSTANCES IN MARINE ORGANISMS

Rovinj, Yugoslavia, 5-9 November 1984

prepared as part of the

Long-term Programme for Pollution Monitoring and Research in the Mediterranean (MED POL Phase II)

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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FAO/UNEP MEETING ON TOXICITY AND BIOACCUMULATION OF SELECTED SUBSTANCES IN MARINE ORGANISMS

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Rome, 1985

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PREPARATION OF THIS REPORT

This report was prepared as part of a cooperative project of the United Nations Environment Programme, entitled:

Mediterranean Action Plan: Support to the Implementation of MED POL-Phase II

with the Food and Agriculture Organization of the United Nations,
the United Nations Educational, Scientific and Cultural Organization,
the Intergovernmental Oceanographic Commission,
the World Health Organization,
the World Meteorological Organization, and
the International Atomic Energy Agency,
as cooperating agencies.

The papers presented at the meeting will be published as a supplement to this report.

DEFINITION OF MARINE POLLUTION

Pollution of the marine environment means: "The introduction by man, directly or indirectly, of substances or energy into the marine environment (including estuaries) which results in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities including fishing, impairment of quality for use of sea water and reduction of amenities".

IMO/FAO/Unesco/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP)

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1. BACKGROUND

The toxicity of certain chemicals reaching the marine environment has aroused public concern which, in turn, has been translated by administrators in many countries into legislative measures designed to prevent marine pollution and consequent incidents.

The Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution have adopted the Dumping and Land-based sources protocols which provide for the prohibition or elimination of pollution from certain substances on the basis of their toxicity, persistence and bioaccumulation.

The Long-term Programme for Pollution Monitoring and Research in the Mediterranean Sea (MED POL - Phase II), which constitutes the scientific/technical component of the Mediterranean Action Plan, is basically divided into two groups of activities, namely monitoring and research. The research component is divided into twelve topics one of which (research activity "G") is concerned with the toxicity, persistence, bioaccumulation, mutagenicity and carcinogenicity of selected substances. In the framework of the monitoring programme, the levels of contaminants (mainly heavy metals and chlorinated hydrocarbons) are periodically measured in selected marine organisms, sea water and sediments.

A meeting, hosted by the Center for Marine Research Rovinj, was jointly convened by FAO and UNEP in the framework of research activity "G". The title of the meeting, for which FAO undertook technical responsibility, was "Toxicity and Bioaccumulation of Selected Substances in Marine Organisms".

The meeting took place in Rovinj, Yugoslavia, from 5-9 November 1984, at the kind invitation of the local Center for Marine Research, "Rudjer Boskovic" Institute, and was attended by 34 participants (see Annex II). Dr. Mirjana Krajnovic-Ozretic was elected Chairman, Professor A.K.H. El-Sebae, Vice-chairman and Dr. P.D. Abel, Rapporteur. Dr. G.P. Gabrielides, FAO Senior Fishery Officer (Marine Pollution), acted as Technical Secretary.

The meeting had the following objectives:

a) to review the progress of the on-going projects undertaken in the framework of research activity "G". The scientists presented their work which was then critically discussed by the meeting. A list of all papers presented is attached as Annex III to this report.

b) to discuss the reliability and interpretation of the current results in relation to natural environmental conditions.

c) to examine the feasibility of developing standard methodology for toxicity experiments.

d) to make recommendations for future research.

The papers presented at the meeting are published in a supplement to this report. The present report which was prepared and adopted by the meeting contains the various discussions, conclusions and recommendations of the meeting.

2. METHODS FOR MEASURING ACUTE LETHAL TOXICITY - NEED FOR STANDARD METHODOLOGY

(This section should be read in conjunction with Annex I.)

Broad general agreement upon appropriate methods for estimating acute lethal toxicity of pollutants to aquatic organisms already exists and is well documented in the scientific literature. (e.g. Sprague, 1969, 1970, 1973; Brown, 1973; Cairns and Dickson, 1973; Mayer and Hamelink, 1977; Marking and Kimerle, 1979; Buikema and Cairns, 1980; Eaton *et al.*, 1980; McLean *et al.* 1980; Bellan, 1981; Branson and Dickson, 1981; Great Britain Standing Committee of Analysts, 1981; OECD, 1981; Alabaster and Lloyd, 1982; Warda and Parrish, 1982). However, the meeting recognised that in many Mediterranean

countries there is a shortage of physical and trained manpower resources. Nevertheless,it was also recognised that certain minimum standards should be adopted and rigorously adhered to in all experiments involving the exposure of aquatic organisms to toxic pollutants in the surrounding medium. The meeting agreed that at the present stage it is neither necessary nor profitable to precisely prescribe the experimental conditions for toxicity tests. However, it was agreed that every laboratory associated with the MED POL programme should seek as soon as possible to adopt scientifically valid methodologies.

Four aspects of toxicity test methodology were identified as being particularly important. These are:

- The test conditions
- The species used in experiments
- The analysis, interpretation and reporting of results
- Special methodological considerations relating to specific pollutants.

2.1 The test conditions

The following conditions are considered essential in any experiment in which aquatic animals are exposed to toxic pollutants in the surrounding medium:

a) The experimental conditions should be such as to allow survival of control organisms for the duration of the experiment. It is recognised that in experiments using, for example, juvenile stages of living organisms it may not be possible to ensure complete survival of the controls. However, where the duration of the experiment is short in relation to the expected lifespan of the organism under test, mortality of control organisms within the duration of the experiment is not acceptable and the use of "correction factors" is scientifically unacceptable. In experiments involving adult or post-larval animals of relatively short duration in relation to their expected lifespan, substantial control mortalities indicate inappropriate experimental conditions, thus invalidating the experiment; and the use of "correction factors" is erroneous. Such "correction factors" may only be appropriately used where the duration of the experiment represents a substantial fraction of the expected lifespan of the animal.

b) Attention should be paid to the effect of the experimental conditions on the concentration of pollutants present in the experimental containers. When any chemical substance is added in relatively low concentrations to a volume of water to which animals are to be added, it is expected that the original concentration will rapidly alter. The concentration of the pollutant can be altered by any of several processes:

   (i) Absorption of the toxic substance by the organisms.

   (ii) Adsorption of the toxic substance onto the walls of the test vessel.

   (iii) Volatilisation of the toxic substance into the air, particularly when the experimental vessel is aerated.

   (iv) Chemical or biological transformation of the toxic substance to forms which may be more or less toxic than the substance originally administered.

   These considerations imply that ideally the water in the experimental vessels should be renewed frequently. It is well established that in practice the test solutions should be renewed or replaced within the

duration of the experiment at a rate which depends upon the physico-chemical properties of the toxic substance being tested (e.g.chemical and biological stability, volatility, etc.). Therefore, it is obvious that laboratories involved in toxicological research should attempt to acquire urgently facilities for carrying out toxicological experiments under continuous-flow conditions. In general, it is required that the toxicant solution is renewed every six to ten hours during the experiment, depending on the properties of the toxic substance and other experimental conditions. There are numerous examples in the literature of methods by which this can be achieved (e.g. Alabaster and Abram, 1965; Mount and Brungs, 1967; Stark, 1967; Burke and Ferguson, 1968; Solon et al., 1968; Abram, 1973; Wuerthele et al., 1973; Bahner et al., 1975; Benoit et al., 1982; Smith and Hargreaves, 1983). Although systems vary in their cost and complexity, many comparatively simple designs are available which require little more than standard laboratory apparatus, and some are specifically designed on a small scale for use with small organisms or where limited space is available.

The meeting recognised that in some laboratories constraints on resources may not afford the development of such facilities in the immediate future. Therefore, it may be necessary to continue to conduct toxicity tests under "static" conditions. In this case, it should be recognised that several precautions are required in order to ensure that the result of the experiment is scientifically valid. Firstly, the volume of the test vessels should be sufficiently large. Detailed recommendations on this point are made by Sprague (1969, 1973), Alabaster and Lloyd (1982) and Ward and Parrish (1982). (See also Annex I). In addition, it is desirable that the test solutions are renewed regularly; the frequency of renewal required again depends upon the properties of the toxic substance and the experimental conditions, but in general renewal of the test solutions should be carried out approximately every 12-24 hours.

c) As the concentration of the toxic substance to which the test organisms are exposed may vary during the experiment (regardless of whether the experiment is carried out under static or continuous-flow conditions) it is highly desirable that the concentration of toxic substance actually present in each experimental vessel is measured frequently during the experiment.

d) Environmental variables which may be expected to influence the toxicity of the toxic substance and/or the physiology of the organisms should also be monitored during the experiments (examples include temperature, pH, salinity, dissolved oxygen concentrations). These results should be reported with the results of the experiment.

e) Proper consideration should be given to matters such as choice of sample size, acclimation of organisms to the experimental conditions, and selection of individuals from stock populations for use in experiments. Such considerations apply to all experiments involving living organisms, not just toxicological experiments; and established scientific conventions on such matters should be followed rigorously. Detailed recommendations on such matters, as they apply to toxicity tests, are given for example, by Sprague (1969, 1973) and by Ward and Parrish (1982).

2.2 The species used in experiments

The choice of species for toxicity tests is subject to the restraints which apply to all biological experiments. Choice is restricted, for example, to those species which can be maintained in laboratory conditions, and which are available in sufficient numbers. Further, the choice of test organism depends upon the purpose for which the

results are required, and the meeting discussed various criteria by which species are selected for investigation. For example, the adoption of a "standard" species is appropriate for some purposes, e.g. where the purpose of the test is to compare toxicity testing procedures employed in different laboratories. However, if the purpose of the experiment is to estimate the potential environmental hazard of a specific pollutant,clearly it is desirable to select a range of species representative of different trophic levels and different taxonomic groups. The meeting recognised that questions relating to the choice of species, or the degree of standardisation required, are not peculiar to toxicological investigations but are common to all biological experiments, and that established scientific practice should be followed, the prime criteria being that the species selected should be appropriate to the purpose of the experiment. These questions as they relate to toxicity experiments have been widely discussed in the literature (e.g. Sprague, 1970; Brown, 1973; Bellan, 1981).

2.3 Analysis, interpretation and reporting of results

The meeting recognised that much confusion is caused by the failure of many investigators to use appropriate methods of data analysis and to clearly report the results. Appropriate methods of data analysis and reporting of results were discussed, and it was agreed that an attempt should be made to meet certain essential requirements.

In lethal toxicity testing, most investigators use the modifications by Litchfield (1949), and Litchfield and Wilcoxon (1949) of the log-probit transform method of Bliss (1935, 1937) to estimate median lethal concentrations (LC50) or median survival times (LT50). The application of this procedure in toxicity testing of pollutants to aquatic animals has been clearly explained by Sprague (1969, 1973). Many Mediterranean workers use Stora's (1972, 1975) application of the Bliss method, which produces acceptable if slightly unconventional results; although since it takes no account of the Litchfield-Wilcoxon modification, computation of results by this method may be unnecessarily time-consuming and tedious. Suitable alternative methods have been discussed by several authors (Sprague, 1969; Stephan, 1977) but in practice appear to have little influence on the final result in most cases.

Results may be expressed in terms of LC50 values for specified times (e.g 24 h, 48 h, 96 h). The limitations of expressing results in this form are well known and have been clearly explained by Sprague (1969). These limitations should be appreciated when the results are interpreted.

A preferable approach is to make frequent observations of mortality in the experimental vessels and to construct a complete toxicity curve. Ideally the experiment should be continued until a lethal threshold concentration is established, as lethal threshold concentrations are a more reliable indicator of lethal toxicity than short-term LC50 values (Sprague, 1969).

The use of computers to assist in the analysis of data was discussed. Computers allow the rapid calculation of results but as there is a danger that undue reliance on their output can allow simple errors to be undetected, manual calculation of representative data from each experiment is therefore recommended as a check.

Results should be presented clearly and in such a way as they can be readily interpreted by other investigators. Particular attention should be paid to the following points:

a) Estimates of error should always be given (e.g. on concentration values), and it should be clearly indicated whether they are in the form of standard deviations, standard errors or 95% confidence limits.

b) Sufficient detail about the experimental methods should be given to aid the reader to evaluate the results. As in any published scientific work, the aim should be to report enough information to allow the experiment to be repeated by an independent investigator.

c) In the discussion of the results, it should be appreciated that the validity of their interpretation depends upon the limitations of the experimental methods. For example, if the experimental conditions are such that the concentration of the toxic substance varies by ±20%, this will influence the degree of precision with which it is appropriate to report the results.

2.4 Special considerations relating to specific pollutants

Some pollutants, because of their physico-chemical properties or their mode of release to the environment, require special consideration to be given to the design of experiments. As examples, two classes of pollutant may be used to illustrate this point.

a) Volatile substances: these substances present a specific difficulty regarding the maintenance of the test concentrations, which referred to in section 2.1 (b). For experiments with small organisms, methods exist for using closed vessels (e.g. Galassi and Vighi, 1981), but experiments with large animals may present particular difficulties which will influence the design of experiments, the constancy of the experimental conditions and the accuracy of the final result.

b) Oil dispersants have several unusual features which influence methods of measuring their toxicity. Firstly, living organisms in the natural environment are normally exposed to high concentrations for short times, so that in this case experiments of short duration are reasonably realistic. Secondly, many recently-developed dispersants are of relatively low toxicity. Since they are immiscible with water, their solubility is frequently less than their toxicity (as 48 h or 96 h LC50). However, since oil dispersants are only released to environments which have been severely polluted with oil, the purpose of testing the lethal toxicity of oil dispersants is to discover whether the dispersant, and the dispersant/oil mixture, are more, or less toxic than the oil alone.

Measuring the toxicity of oil, oil dispersants and oil/water/dispersant emulsions presents many difficulties, including for example that the toxicity of the toxic substance depends crucially upon the nature of the emulsion; thus in practice the result of a toxicity experiment depends largely upon the way in which the dispersant, oil, or dispersant/oil mixture is mixed with the water. Additionally, oil is not a precisely definable substance, but a variable mixture of a large number of separate compounds. However, several approaches to the measurement of oil dispersant toxicity have been developed in various parts of the world and appropriate methods are described in detail by, for example, Laroche et al., (1970); McCarthy et al. (1973, 1978), Beynon and Cowells (1974), Blackman et al. (1977), Thompson and Wu (1981) and Moldan and Chapman (1983).

These examples illustrate that certain toxic substances have particular physico-chemical characteristics which should influence the choice of test methods. In general, consideration should always be given to the physico-chemical properties of the pollutant in the design, conduct and interpretation of toxicity tests.

N.B. Annex I contains some methodological suggestions and examples for acute toxicity tests on marine organisms for screening purposes.

3. OBJECTIVES OF TOXICITY TESTING

Toxicological experiments and observations are carried out for various reasons; within the Mediterranean area three distinct types of toxicological investigation may be identified as important:

a) Rapid screening tests for legal/administrative purposes.

b) Research investigations designed, for example, to identify the actual or potential environmental hazards associated with specific pollutants, or to assist in formulating water quality standards.

c) Investigations designed to monitor the toxic effect of pollutants on natural populations.

3.1 Rapid screening tests

In many countries permission to discharge an effluent, or to use a particular oil dispersant, is conditional upon a measure of toxicity. Thus, if the toxicity of the effluent or dispersant exceeds a certain limit (however that is defined), permission to discharge it to the environment is denied. A test for this purpose must fulfill the following requirements:

a) The method must be clearly defined and rigorously followed. Since the result of the test is required for legal or administrative purposes, and its result may have economic implications (e.g. in the case of an oil dispersant which "fails" the test) the result of the test may be challenged. Because variations in experimental conditions can sometimes have a large influence on the results, there is a danger that an interested party may challenge the results on the grounds that the test procedure was not properly followed.

b) Subject to the minimum standards of methodology outlined and recommended in this report, the test should be as simple as possible. This is firstly because large numbers of such tests may need to be carried out, and secondly because they may need to be carried out with limited laboratory facilities.

c) For similar reasons, the test should be of relatively short duration.

Screening test methods have been developed in many countries. Tests for oil dispersants have been referred to above. Among tests for more general purposes, the best known is the APHA/AWWA/WPCF (1980) and the OECD (1981) methods. Details of the screening tests used in several countries, for freshwater fish, are given by EIFAC (1983) and Alabaster and Lloyd (1982). Toxicity tests for marine organisms are described by Persoone et al. (1984, 1984a).

3.2 Research investigations

Toxicological investigations are also needed in order to:

a) identify actual or potential environmental hazards associated with particular pollutants. This approach could be of particular interest if referred to new chemical compounds introduced to the natural environment and must follow acute toxicity screening tests aimed to obtain a rough ranking of the potential hazard, as prescribed by several international legislations as for example the 6th amendment of the EEC Directive 79/831 on Dangerous Substances.

b) to contribute to the establishment of "safe" or environmentally acceptable levels of water pollutants, i.e. to the development of water quality standards.

This would include, for example, study of the effects of environmental factors (e.g. salinity in relation to brackish or estuarine water discharges, or temperature effects in relation to discharge of heated effluents) on toxicity and bioaccumulation; the toxicity of pollutants in combination; and the possibility that organisms inhabiting polluted areas may have become genetically adapted or physiologically acclimated to specific pollutants.

To meet this objective a wide variety of experimental approaches need to be employed. Therefore, it is inappropriate to attempt to define "standard" methodologies in terms, for example, of the species to be tested, or the environmental conditions. In research designed for these purposes the only standards which are required are those of established sound scientific practice.

Subject to these considerations a variety of approaches may be considered valuable. Acute lethal toxicity tests remain valuable, particularly within the context of sequential hazard assessment schemes such as are described by Duthie (1977), Maki and Duthie (1978) and Tooby (1978). However, it should be appreciated that the application of the results of acute lethal toxicity tests to the assessment of environmental hazards is only scientifically valid if certain additional criteria (for example, relating to the test duration and the estimation of lethal threshold concentrations) are met. This point has already been referred to in section 2.3.

Additionally, the value of long-term experiments to investigate the effects of sublethal levels of pollutants, particularly on biological variables of ecological significance (e.g. growth, reproduction and development) is generally recognised. Other approaches which have been shown to be of value include the use of controlled or partially-controlled ecosystems both in the laboratory and in the field. Similarly, studies of bioaccumulation of pollutants in the field and in the laboratory are profitable, again provided that they take place within the context of a clearly defined programme of hazard assessment. Nevertheless, it should be recognised that in the Mediterranean region, the contribution of toxicological research to the monitoring and control of pollution is for practical purposes exactly the same as it is in other regions of the world. Therefore, there is no good scientific reason why completed and published research originating from other regions of the world should not be adapted to the problems of the Mediterranean. Indeed, it can be argued that much of the research currently undertaken in the Mediterranean countries unnecessarily duplicates research carried out several years ago in other parts of the world for exactly the same purpose. For example, the lethal toxicity of common pollutants such as heavy metals and pesticides to marine species is well documented. There is no reason whatsoever to suppose that, in general, Mediterranean species differ significantly in their sensitivity to pollutants from species in other parts of the world. Indeed, in some cases, the toxicological literature of the last 20 years indicates that interspecific and intraspecific differences in susceptibility to pollutants is relatively small. It can therefore be argued that the measurement of the lethal toxicity of common pollutants such as heavy metals and many pesticides to Mediterranean species does not necessarily represent the most effective use of physical and manpower resources, which could be directed to more effective ends.

3.3 Monitoring of toxic effects of pollutants on natural populations

Bioaccumulation of pollutants in aquatic organisms is an important aspect of toxicological research since clearly pollutants can exert no effect unless they enter the tissues of living organisms. Consequently, much effort has been put into the monitoring of pollutant levels in the tissues of living organisms from natural populations, partly from the point of view of the control and monitoring of pollution and partly because of the possible public health implications. Thus, a considerable volume of information has been acquired on the levels of pollutants in the tissues of species in many areas of the Mediterranean. However, it is important to establish the relationship between measured pollutant levels in plant and animal tissues and the existence of toxic effects which have ecological significance. Potentially useful approaches to this question include several areas of investigation. Long-term studies on the population dynamics of species in polluted and unpolluted areas of the Mediterranean are required to establish whether populations inhabiting polluted areas

are being ecologically affected by the prevailing levels of pollution. For example, studies of the population density, age structure, growth rate and reproductive success of selected species in conjunction with continuing studies on bioaccumulation may be profitable. In addition, controlled ecosystem studies both in field and laboratory conditions are potentially useful.

Obviously, however, investigations of this kind are both time-consuming and expensive and there would appear to be advantages in investigating the use of more rapid methods of detecting sublethal toxic effects in occurring natural populations exposed to pollution. There are several recent developments which offer the possibility of identifying sublethal toxic effect in natural populations exposed to pollutants. Examples include the use of tissue metallothionein content to indicate exposure of organisms to heavy metal pollution; (e.g. Howard and Nickless, 1975; Olafson et al., 1979; Roesijadi, 1980; Roch et al., 1982; Roch and McCarter, 1984); the use of genotoxicological techniques (Kohn et al., 1976; Zahn et al., 1981, 1982, 1983; Hoehn-Bentz et al., 1983; Kurelec et al., 1983) and the use of mixed function oxidases as an indicator of exposure to PAH and PCB (Nebert and Gelboin, 1968; Payne and Penrose, 1975; Kurelec et al., 1977; Kezic et al., 1983). Some laboratories in the Mediterranean area have already begun to apply these techniques. However, it is recognised that in spite of their great potential usefulness, they require extensive facilities in terms of physical resources and skilled manpower. Consideration should perhaps be given to the possibility of further investigating the application of such approaches in the Mediterranean area.

4. BIOACCUMULATION OF CONTAMINANTS IN MARINE ORGANISMS

A variety of factors can affect the bioaccumulation of contaminants in marine organisms.

The temperature of the ambient water influences the metabolic rate of the organisms and hence the uptake and release of contaminants. Some differences in contaminant concentrations found in the same species at different seasons may be attributable to temperature differences which affect fat metabolism. If dissolved oxygen values are low enough to affect respiratory processes, bioaccumulation of contaminants may be influenced. Although in sea water pH is in general very constant, local variations can occur in particular areas. This may influence bioaccumulation indirectly, since pH is a major determinant of the degree to which some pollutants, particularly metals, form inorganic or organo-metal complexes, thus influencing the biological availability of the contaminant. Salinity variations, where they occur, can also affect bioaccumulation indirectly, since adaptation to abnormal salinity can involve extensive metabolic adjustment.

In addition to the abiotic factors mentioned above, bioaccumulation can be influenced by the biological state of the organism. For example, correlations are frequently found between contaminant concentrations in tissues and the age or size of the organism. Differences in contaminant concentrations in whole organisms or in specific tissues may also occur at different stages of the life cycle. For example, before the formation and ripening of gonads in fish, food is stored as fats in the liver and other parts of the body. This could influence the storage of organic and organometal compounds, many of which have a high affinity for lipids. Before and during the reproductive period, food reserves are mobilised and redistributed, and this will clearly influence the levels of contaminants detected in particular tissues, e.g. gonads. This is one reason for the high variability observed in reported results of the levels of contaminants in many species.

Based on the above, the meeting recommends that the following should be taken into consideration when the levels of pollutants are monitored in marine organisms:

a) Relevant information on environmental conditions (e.g. temperature, salinity, dissolved oxygen, levels of organic matter etc. as appropriate) should be collected. Preferably such information should cover not only the time of sampling, but if possible the period preceding the sampling.

b) The following biological information should be collected: length, weight, sex and maturity of the specimens. If possible, the age of the specimens should be estimated.

c) An effort should be made to analyse not only the flesh but also other tissues and organs (e.g. liver, gonads) as appropriate.

d) As sediment and/or food will be present in the alimentary tract of animals, this should be removed before analysis.

e) Individual as well as composite samples should be used for analysis. Composite samples should be made up of at least 10 individuals of the same size. For individual samples a minimum of 10 specimens is also recommended.

f) Consideration should be given to the possible influence of reproductive activity on the results of analyses. For example, where possible samples should be taken before, during and after spawning; and wherever possible the reproductive status of the specimens should be noted.

5. CONCLUSIONS

The validity of the results of toxicological investigations is greatly influenced by the methods employed. Partly due to shortages of skilled manpower and physical resources in some laboratories, the results of many investigations are of doubtful value.

Certain minimum methodological requirements may be agreed upon, to which all laboratories engaged in toxicological research should adhere. Research should be encouraged only where it is established that these requirements can be met, and institutions which cannot meet them should be encouraged to develop their ability to do so.

Many toxicological investigations unnecessarily duplicate research being carried on elsewhere, or already completed and published. However, it is possible to identify several specific objectives for toxicological research within the Mediterranean area, and research which seeks to meet these objectives should be encouraged.

Beyond the basic methodological requirements, the degree of standardisation of methodology which is desirable depends upon the objectives of the study. As far as it is practicable, a standard approach to screening tests for legal and administrative purposes, for example, has some advantages and should be encouraged. Some toxicological investigations are designed to contribute to assessment of environmental hazard or to establish water quality standards. In these cases a more diverse approach is desirable, provided that the minimum standards of methodology are met.

Specific areas of research have been identified as being of particular value within the Mediterranean context, and participating laboratories should be encouraged to develop research in these areas (see sections 3.1 and 3.2).

Some individual laboratories are developing facilities and expertise in specific techniques, and this should be encouraged.

It is likely for the foreseeable future that laboratories will continue to differ widely in their levels of technical competence. For every laboratory to seek to achieve parity with others, in terms for example of expensive and specialised equipment, may not represent the most effective use of limited resources. Further, many lines of research have been identified as desirable which are beyond the capability of a single laboratory. These considerations indicate that collaboration between institutions should be encouraged, not only in research but also in the training of scientific and technical staff.

Support for research proposals should depend upon whether the proposal meets certain criteria identified in this report. These are that acceptable standards of methodology are employed, and that the proposal should be clearly shown to be meeting one or more of the objectives listed in section 3.

A great deal of data has been obtained on the concentration of pollutants in the tissues of plants and animals of the Mediterranean. The value of this data is limited for several reasons. Factors such as the size, age, physiological status, stage of the reproductive cycle and environmental conditions are known to have large effects on the metabolism and distribution of metals and other toxic substances in marine organisms. However, the relevant data are not always collected in conjunction with the sampling of the organism. Consequently, it is frequently unclear, for example, whether differences in pollutant levels in organisms from different areas are caused by different levels of environmental contamination, or by other factors.

6. RECOMMENDATIONS

On the basis of the above conclusions the meeting recommended that:

a) laboratories should be encouraged to develop the technical facilities to enable them to employ appropriate methods of research, so that the quality and reliability of results is improved.

b) there is a need for standardisation of toxicity testing procedures with marine organisms for screening purposes, involving substances such as oil dispersants and some others, where the results may be of legal or administrative, rather than strictly biological significance. FAO should produce a document describing in detail recommended methods for screening tests, similar to that produced by the OECD for freshwater organisms in the Guidelines for Testing of Chemicals.

c) a diversity of approaches to toxicity studies (e.g. acute and chronic toxicity testing, controlled ecosystem studies, bioaccumulation) on a variety of species is desirable; but care should be taken to ensure that research is not unnecessarily duplicated.

d) preference should be given to investigations which resemble field conditions, or which can be more readily applicable to field conditions, e.g. in the context of a sequential hazard assessment scheme.

e) attempts should be made to determine whether observed levels of pollutants in field populations are associated with the occurrence of sublethal toxic effect (e.g. by long-term ecological studies, controlled ecosystem studies, or the use of biochemical, physiological, immunotoxicological or genotoxicological techniques).

f) attention should be paid to the substances listed in the Annexes to the Protocols of the Barcelona Convention, on which inadequate information is available.

g) collaboration between institutions on specific research projects should be encouraged as many important investigations are beyond the resources of a single laboratory.

h) collaboration between institutions on training of scientific and technical staff is desirable. Encouragement should be given to individuals or small groups to visit other participating laboratories to gain practical experience of specific techniques.

i) agreement should be reached between institutions and between chemists and biologists, on detailed methods for sampling of biological materials and analysis of tissues for pollutants.

Recommendations a), g) and h) are regarded as particularly important.

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Annex I

METHODOLOGICAL SUGGESTIONS AND EXAMPLES FOR ACUTE TOXICITY TESTS ON MARINE ORGANISMS FOR SCREENING PURPOSES

N.B. Methodological suggestions included in this annex are only recommended provided that the general principles outlined in Section 2 of the main report are also followed.

Organisms

Toxicity tests could be made on representative organisms of three different trophic levels: primary producers, primary consumers and secondary consumers (mainly fish).

The following characteristics are important in the choice of the test organisms:

- easy to obtain throughout the year;
- easy to maintain in the laboratory under controlled conditions;
- easy to identify taxonomically to species;
- well known from the biological point of view; i.e. there should exist adequate information on their normal physiology, ecology, life history etc.;
- it is desirable that genetically homogenous material is used;
- if possible, sensitive early life stages should be used;
- if possible, cosmopolitan organisms should be used, i.e. species which are widely distributed throughout the Mediterranean.

a) Primary producers

Several planktonic algae are currently used for testing in standard conditions. For example, the chlorophycean Dunaliella tertiolecta, and the diatoms Phaeodactylum tricornutum and Skeletonema costatum can be easily obtained in pure culture from botanical and marine biological institutions. A simple method to cultivate them is reported in the Marine Algal Assay Procedure (US EPA, 1974).

The procedure for toxicity testing could be similar to those proposed for the freshwater algae in the OECD Guidelines for Testing of Chemicals (OECD, 1981).

b) Primary consumers

Several different marine invertebrates could be proposed, for example:

- Artemia salina;
- planktonic copepods or other phytophagous crustaceans;
- molluscs;
- sea urchins.

Methods for breeding and toxicity testing with Artemia are well known. For other animals it is necessary to establish clearly responses and reactions.

Homogeneous developmental stages and size classes must be used.

If animals from natural environment are used they must be taken from unpolluted areas.

c) Fish

Young specimens, 6-8 cm length, of mullets (genus Mugil or Liza) are commonly used in toxicity tests. Other species could be used if they possess the desirable general characteristics for test organisms. Commercial species from aquaculture (i.e. Dicentrarchus labrax) could also be very useful.

Experimental Conditions

Both synthetic sea water and natural sea water taken from unpolluted areas are acceptable for toxicity experiments.

Natural sea water may need filtering to avoid particulate matter and natural planktonic populations, especially if water is used for algal tests. When using synthetic sea water, care should be taken to ensure that it is adequately equilibrated with air before use, and that any precipitate formed is removed by filtering. The ratio between water and organisms should not exceed 0.5g of organism (wet weight) per liter of water.

Salinity must be controlled and reported and should normally be around the levels typical for the Mediterranean (38 o/oo S).

Temperature should be controlled and recorded during the experiment, and preferably should not vary by more than $\pm 1^{o}$ C. Temperature conditions must be clearly reported with the results.

Depending upon the type of test and of organism, light could be continuous, light-dark cycle or dark. The conditions of light must be reported.

The oxygen content must be measured daily and must be at least 70% of the saturation throughout the experiment.

If artificial sea water is used the pH must be controlled and must be 8.2 (± 0.2). This pH could be adjusted in general by aeration.

The concentration of the tested chemical must be at least 80% of the initial concentration throughout the experiment. The concentration of the chemical must be preferably measured regularly during the experiment.

Devices able to maintain a concentration above the level of 80% must be adopted (continuous or semi-continuous flow if necessary).

Organisms must be acclimated in the water used for the tests for an appropriate period according to the type of organism (i.e. for planktonic crustaceans two days, for fish at least five days).

During acclimation period mortality must be recorded.

Animals should normally not be fed 24 hours before the experiments or during the experiments.

Annex II

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Annex III

LIST OF PAPERS PRESENTED

1. Abel P.D. and E. Papathanassiou

   "Effects of toxic pollutants on the filtration rate of a Mediterranean bivalve mollusc."

2. Balci A.

   "Toxicity and bioaccumulation of PCBs in marine organisms."

3. Belkhir M.

   "Oil dispersant toxicity to fish and molluscs."

4. Calamari D. and M. Vighi

   "The role of Log P in predicting bioconcentration and toxic potential of organic chemicals for aquatic organisms."

5. Castritsi-Catharios J. and V. Kiortsis

   "Acute toxicity of an oil dispersant to Artemia Salina."

6. Catsiki A.V.

   "The variability in levels of copper and zinc in the tissues of selected species from the Berre lagoon (France)."

7. Dalla Venezia L. and V.U. Fossato

   "Effects of PCBs in Leander adspersus: toxicity, bioaccumulation, oxygen consumption, osmoregulation."

8. El-Rayis O. A.-M. and A.A. Ezzat

   "Bioaccumulation of some heavy metals in coastal marine animals in the vicinity of Alexandria. I: Bio-assay."

9. El-Rayis O. A.-M. and A.A. Ezzat

   "Bioaccumulation of some heavy metals in coastal marine animals in vicinity of Alexandria. II: Surveying."

10. El-Rayis O. A.-M.

    "Bioaccumulation of cadmium in plankton, bivalves, crustacea and in different organs of six fish from Mex Bay, west of Alexandria."

11. El-Sebae A.H., M.A. El-Amayem, I. Sharaf and M. Massod

    "Factors affecting acute and chronic toxicity of chlorinated pesticides and their biomagnification in Alexandria region."

12. Krajnovic-Ozretic M. and B. Ozretic

"Toxicity testing in the marine environment."

13. Martic M., N. Ajdacic, J. Stjepcevic and M.J. Gasic

"Investigation of trace element distribution in the aquatic system of the Boka Kotorska Bay."

14. Moraitou-Apostolopoulou M. and G. Verriopoulos

"Lethal and sublethal effects of some contaminants (heavy metals, oils, dispersants) to marine planktonic animals."

15. Obersnel V. and C. Lucu

"Effects of Cadmium on spermatozoa and fertilized eggs of sea urchins Paracentrotus lividus Lam."

16. Ozretic B. and M. Krajnovic-Ozretic

"Sea urchin gametes and their developing embryos in marine toxicology studies."

17. Papathanassiou E.

"Effects of cadmium ions in the process of Oogenesis of the common prawn Palaemon serratus (Pennant)."

18. Pavicic J., M. Skreblin, I. Kregar, M. Tusek-Znidaric and P. Stegnar

"Determination of Cd-binding proteins similar to metallothionein in the digestive gland of Mytilus galloprovincialis with regard to a preliminary treatment of the sample.

19. Saleh H.H. and A. Hamza

"Study on the health condition of Tilapia zillii Gerv. surviving in the polluted water of Lake Mariut, Alexandria (Egypt)."

20. Tudor M. and I. Katavic

"Acute toxicity of an oil dispersant to the developmental stages of the sea bass Dicentrarchus labrax."

21. Tusek-Znidaric M., M. Skreblin, J. Pavicic, I. Kregar and P. Stegnar

"Mercury-binding proteins of the gills and digestive gland of Mytilus galloprovincialis."

22. Unsal M.

"Uptake, accumulation and toxicity of vanadium and tin in marine organisms."

23. Zahn R.K.and B. Kurelec

"Genotoxic risk assessment."

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